

Ethylene Production from Methionine

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1. A new reaction is described in which ethylene is formed from the Cu^+ -catalysed breakdown of methionine in phosphate buffer at 30° in air. Some of the other products of the reaction are methionine sulphone, methionine sulfoxide, homocysteic acid, homocystine, acrolein, dimethyl disulphide, methanethiol, ethyl methyl sulphide, methane and ethane. These are considered to be produced in different reaction pathways. 2. Hydrogen peroxide is an intermediate in this reaction and can support ethylene production in the model system in anaerobic atmospheres. Cuprous copper is the active form that catalyses the formation of ethylene from an oxidized intermediate. The initial reaction is probably a Strecker degradation, but the aldehyde product is further degraded to ethylene and other products. 3. Methional ($\text{CH}_3\cdot\text{S}\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{CHO}$) is the most effective producer of ethylene in the model system and appears to be an intermediate in the reaction. 4. The evidence, from both tracer studies and from other precursors of ethylene in the reaction, indicates that ethylene is derived from the $-\text{CH}_2\cdot\text{CH}_2-$ group of methionine.

The formation of ethylene in a model system in which Cu^+ catalyses the breakdown of peroxidized linolenic acid has been described (Lieberman & Mapson, 1964). It was proposed that the biosynthesis of ethylene may proceed through an analogous system catalysed by a copper enzyme. Recent experiments, however, suggest that ethylene in tissues may arise from more than one source. For example, an analysis of particulate fractions, isolated from apples, shows that at least two types of compounds are present which can yield ethylene in the Cu^+ -catalysed system. The first is associated with the fat-soluble constituents of the cell and the second with the water-soluble constituents.

Search for other precursors of ethylene has led to the finding that L-methionine, in reaction with the cuprous generating system, also forms ethylene. It is shown below that methionine and related substances are unique in their ability to form relatively large quantities of ethylene in a model system. The present paper describes characteristics of this ethylene-forming system, demonstrates that ethylene is derived from C-3 and C-4 of methionine, and suggests a mechanism for the reaction.

MATERIALS AND METHODS

Model system. The model system consisted of L-methionine (1 mM), Cu^{2+} (1.0 or 0.1 mM), ascorbate (10 mM) and phosphate buffer, pH 6.8 (60 mM). Components of the

model system were contained in 25 ml. flasks sealed with one-hole rubber stoppers containing clamped capillary tubes. The flasks were incubated at 30° in a water bath-shaker and internal atmospheres above the reaction mixture were sampled periodically with gas-tight syringes. Corrections were made to account for the increase in gas volume resulting from this sampling technique.

Gas analysis. Gases produced in the reaction were determined by gas chromatography. Alumina or silicone (30% silicone oil on Celite) columns and a flame-ionization detector were used to determine ethylene. Details of the complete system have been described by Meigh, Norris, Craft & Lieberman (1960). Acrolein, ethyl methyl sulphide, dimethyl sulphide, dimethyl disulphide and methanethiol were detected on a capillary column (80 ft. \times 0.015 in. diam.) at 15° with di-(2-cyanoethyl) ether as stationary phase, with an enrichment trap cooled in liquid O_2 to first concentrate the gases. The vapours were subsequently released from the trap by passing an electric current through the trap.

Methional. This compound was prepared from acrolein and methanethiol by the method described by Pierson, Giella & Tishler (1948).

Tracer experiments. The tracer studies were carried out with L-[Me- ^{14}C]methionine, DL-[carboxy- ^{14}C]methionine, DL-[2- ^{14}C]methionine, or DL-[3,4- $^{14}\text{C}_2$]methionine, added to the reaction mixture of the model system. The reactions were carried out in Warburg flasks containing side arms with and without 10% (w/v) KOH. The difference in counts between flasks with and without KOH was attributed to CO_2 formed in the reaction. A 2 ml. sample of the gases evolved in these reactions was first assayed on the gas chromatograph, and then about 50% of the gaseous

atmosphere in the reaction flask was removed with a 50 ml. gas-tight syringe for ^{14}C analysis. This gaseous sample was transferred to a Thunberg tube containing 15 ml. of ice-cold $\text{Hg}(\text{ClO}_4)_2$ solution, which on shaking absorbed practically all the ethylene in the gaseous sample (Young, Pratt & Biale, 1953). Residual gases not absorbed by $\text{Hg}(\text{ClO}_4)_2$ solution were transferred by syringe to another Thunberg tube containing 20 ml. of cold toluene with 0.4% of 2,5-diphenyloxazole and 0.01% of 1,4-bis-(5-phenyloxazol-2-yl)benzene as fluorescing agents. These gases were absorbed in the toluene mixture, which was subsequently transferred to counting vials. The $\text{Hg}(\text{ClO}_4)_2$ solution containing the absorbed ethylene was transferred to still another Thunberg tube and the absorbed ethylene released with HCl in a closed system. The ethylene released was transferred by gas-tight syringe to a Thunberg tube, containing the toluene mixture, and absorbed into the toluene by shaking. In preliminary experiments with $[^{14}\text{C}]$ ethylene, absorption and subsequent release from $\text{Hg}(\text{ClO}_4)_2$ solution allowed recovery of more than 95% of the standard $[^{14}\text{C}]$ ethylene. Radioactivity due to ^{14}C was determined in a Packard Tri-Carb liquid-scintillation counter. Counting efficiency was approx. 50% and backgrounds were about 10 counts/min. Parallel experiments were carried out with unlabelled methionine as controls.

Further experimental details are described in the Tables, Figures and text where applicable.

RESULTS

Model system

We have found that D- or L-methionine is an effective substrate for ethylene production in a model system in which it is incubated at 30° with Cu^{2+} and ascorbate in phosphate buffer. The pH optimum for this system is about 7.0 (Fig. 1). If we assume that 1 mol. of ethylene is formed from 1 mol. of methionine our model system converts approx. 2.5% of the methionine substrate into ethylene in 30 min. High (10 mM) and low (less

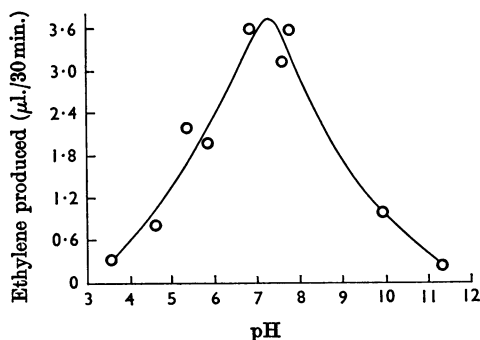


Fig. 1. pH-activity curve for the model system. Flasks contained acetate or phosphate buffer (60 mM), methionine (1 mM), Cu^{2+} (0.1 mM) and ascorbate (10 mM) in a total volume of 5 ml. Flasks were incubated at 30° for 30 min. with shaking.

than 0.1 mM) concentrations of Cu^{2+} depress ethylene production in this system, when compared with Cu^{2+} concentrations of 0.1 mM and 1 mM, with ascorbate at 10 mM in all cases (Fig. 2). It is also clear that concentrations of methionine greater than 0.1 mM are necessary for the significant production of ethylene. A methionine/ Cu^{2+} molar ratio 10–100 appears to be optimum for ethylene formation in this system. Concentrations of ascorbate were varied in the range 5–15 mM without altering ethylene production to any great extent.

When ascorbate is omitted from the model system virtually no ethylene is produced. There is also no ethylene formed when Cu^{2+} is omitted from the system. However, some ethylene is produced, in the absence of ascorbate, when Cu^+ is substituted for Cu^{2+} in the model system (Table 1). These results suggest that Cu^+ catalyses the degradation of methionine, and that ascorbate serves as a reducing agent that converts Cu^{2+} into the active

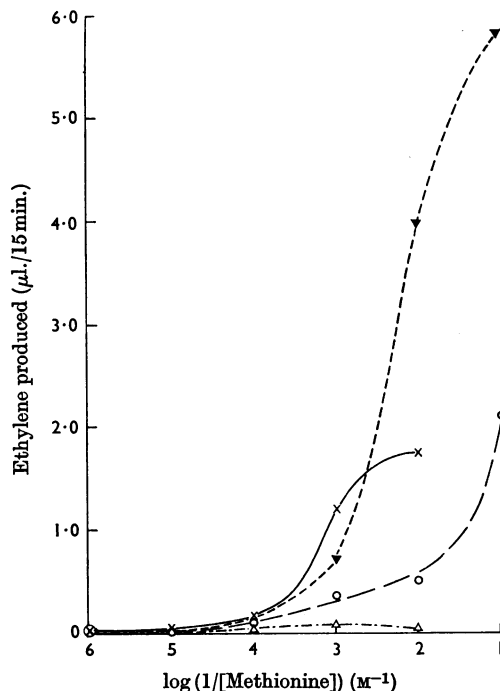


Fig. 2. Ethylene production related to methionine concentration at different concentrations of Cu^{2+} , with ascorbate held constant at 10 mM. These experiments were run for 15 min. to ensure a sufficient concentration of ascorbate for the reduction of Cu^{2+} to Cu^+ at all the concentrations used. The reaction mixtures contained phosphate buffer, pH 6.8 (60 mM). The concentrations of Cu^{2+} used were: ○, 10 mM; ▼, 1 mM; ×, 0.1 mM; △, 0.01 mM. The flasks were incubated at 30° with shaking.

Table 1. *Ethylene formation in the model system under various conditions*

Flasks contained phosphate buffer, pH 6.8 (60mm), methionine (1mm) and, where indicated, ascorbate (10mm) and Cu^{2+} or CuCl (0.1mm) in a total volume of 5ml. Flasks were incubated at 30° for 30min. with shaking. The CuCl was dissolved in deoxygenated saturated KCl .

Flask contents	Ethylene produced ($\mu\text{l./30 min.}$)
(1) Phosphate buffer + methionine + Cu^{2+}	0.00
(2) Phosphate buffer + methionine + ascorbate	0.06
(3) Phosphate buffer + methionine + Cu^{2+} + ascorbate	3.40
(4) Phosphate buffer + methionine + Cu^{+}	0.23

Cu^{+} . Since methionine is the only carbon source, in the absence of ascorbate and in the presence of Cu^{+} , the ethylene formed must derive from methionine.

Other metal ions. A number of metal cations were tested as substitutes for Cu^{2+} or Cu^{+} in the model system but only Cu^{2+} or Cu^{+} was found to catalyse the reaction. However, when hydrogen peroxide (10–17mm) was added to the model system, Fe^{2+} was also effective as a catalytic agent for the degradation of methionine to ethylene. Approx. 20–60% as much ethylene was formed by the Fe^{2+} system with hydrogen peroxide as by the comparable Cu^{2+} system. Co^{2+} , Mg^{2+} , Pb^{2+} , Zn^{2+} and Mn^{2+} were completely ineffective as substitutes for Cu^{2+} , when care was taken to exclude contaminating Cu^{2+} . The action of Cu^{2+} is thus unique in the model system, in that it does not require exogenous hydrogen peroxide to degrade methionine to ethylene.

Intermediate reactants of the model system

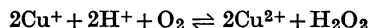
Hydrogen peroxide as intermediate. In a nitrogen atmosphere the usual model system (containing methionine, Cu^{2+} and ascorbate) is inoperative (Expt. 1 in Table 2). However, the introduction of hydrogen peroxide into the system induces a large increase in ethylene production, which reaches a level twice that obtained by the usual system in air. The increase of ethylene production in the model system, induced by hydrogen peroxide, occurs in both air and nitrogen atmospheres and in systems containing either Cu^{2+} or Cu^{+} . It is especially marked in the Cu^{+} system in nitrogen, which contains no ascorbate. Because of the marked stimulatory effect of hydrogen peroxide, and the fact that catalase and peroxidase can almost completely inhibit ethylene production in the model system (Expt. 2 in Table 2), there is a strong

Table 2. *Evidence for involvement of hydrogen peroxide in the methionine model system*

Flasks contained phosphate buffer, pH 6.8 (60mm), methionine (1mm), ascorbate (10mm) and Cu^{2+} or CuCl (0.1mm) in a total volume of 5ml. Where used, the concentration of H_2O_2 was 17mm. Flasks were incubated at 30° for 30min. with shaking.

Flask contents	Atmosphere ($\mu\text{l./30 min.}$)	Ethylene produced
Expt. 1		
(1) Phosphate buffer + methionine + Cu^{2+} + ascorbate	N_2	0.0
(2) Phosphate buffer + methionine + Cu^{+} + ascorbate	N_2	0.03
(3) Phosphate buffer + methionine + Cu^{+} + H_2O_2	N_2	11.1
(4) Phosphate buffer + methionine + Cu^{2+} + ascorbate + H_2O_2	N_2	6.7
(5) Phosphate buffer + methionine + Cu^{2+} + ascorbate	Air	2.6
(6) Phosphate buffer + methionine + Cu^{2+} + ascorbate + H_2O_2	Air	5.4
Expt. 2		
(1) Phosphate buffer + methionine + Cu^{2+} + ascorbate	Air	2.8
(2) Phosphate buffer + methionine + Cu^{2+} + ascorbate + catalase (29000 i.u.)	Air	0.05
(3) Phosphate buffer + methionine + Cu^{2+} + ascorbate + horse-radish peroxidase (24000 i.u.)	Air	0.0

indication that hydrogen peroxide is an intermediate reactant in the model system. Production of hydrogen peroxide during the reaction is assumed to arise after the formation of Cu^{+} by the reduction of Cu^{2+} with ascorbate (Mapson, 1941) as follows:



Hydrogen peroxide as initiator of the reaction. The production of ethylene from methionine may be initiated with hydrogen peroxide in the absence of ascorbate but in the presence of Cu^{2+} [(8) and (9) in Table 3]. Under these conditions, there is a rapid production of ethylene that falls off with time and can be revived and accelerated by the addition of ascorbate (10mm) or Cu^{+} (10mm) (Fig. 3). However, Cu^{2+} at 10mm is ineffective in reviving the reaction. The inhibitory nature of Cu^{2+} , in the model system, when its concentration is ten times that of methionine, has been noted above (Fig. 2), but it appears that Cu^{+} is not inhibitory at this concentration.

Hydrogen peroxide can also initiate ethylene production in the apparent absence of added Cu^{2+} .

Table 3. *Initiation of ethylene reaction with hydrogen peroxide in the presence and absence of Cu^{2+} or ascorbate*

Flasks contained phosphate buffer, pH 6.8 (60 mM), methionine (1 mM) and, where indicated, H_2O_2 (17 mM), Cu^{2+} (1 mM), ascorbate (10 mM) and EDTA (0.1 mM) in a total volume of 5 ml. Flasks were incubated at 30° for 30 min. with shaking. Water was doubly distilled and passed through an ion-exchange column.

Flask contents	Atmosphere	Ethylene produced ($\mu\text{l./30 min.}$)
(1) Phosphate buffer + methionine + H_2O_2	Air	0.00
(2) Phosphate buffer + methionine + H_2O_2	N_2	0.06
(3) Phosphate buffer + methionine + H_2O_2 + ascorbate	Air	2.21
(4) Phosphate buffer + methionine + H_2O_2 + ascorbate	N_2	2.32
(5) Phosphate buffer + methionine + H_2O_2 + ascorbate + EDTA	Air	0.70
(6) Water + methionine + H_2O_2 + ascorbate	Air	0.09
(7) Water + methionine + H_2O_2 + ascorbate + EDTA	Air	0.00
(8) Phosphate buffer + methionine + H_2O_2 + Cu^{2+}	Air	2.32
(9) Phosphate buffer + methionine + H_2O_2 + Cu^{2+}	N_2	3.07
(10) Phosphate buffer + methionine + H_2O_2 + Cu^{2+} + ascorbate	Air	5.48
(11) Phosphate buffer + methionine + H_2O_2 + Cu^{2+} + ascorbate	N_2	6.74

In this case, the presence of ascorbate is necessary [(1) and (3) in Table 3]. The latter reaction is almost entirely due to contaminating metal ions, presumed to be Cu^{2+} introduced by phosphate buffer, since EDTA can markedly decrease ethylene production in this system [(5) in Table 3]. The extent of ethylene production when only contaminating amounts of metal ion (Cu^{2+}) are present (at about $0.1 \mu\text{M}$) indicates the much greater sensitivity of the system to Cu^{2+} with exogenous hydrogen peroxide. The amount of ethylene formed in the reactions initiated by hydrogen peroxide, in systems lacking either ascorbate or added Cu^{2+} [(3) and (8) in Table 3], is approximately equal to the ethylene formed in the model system [Expt. 1 (5) in Table 2] without added hydrogen peroxide. The systems lacking either ascorbate or Cu^{2+} are referred to below as 'abbreviated' systems.

In the absence of ascorbate a Cu^{2+} concentration of at least 1 mM is required when the ethylene-forming reaction is initiated by hydrogen peroxide.

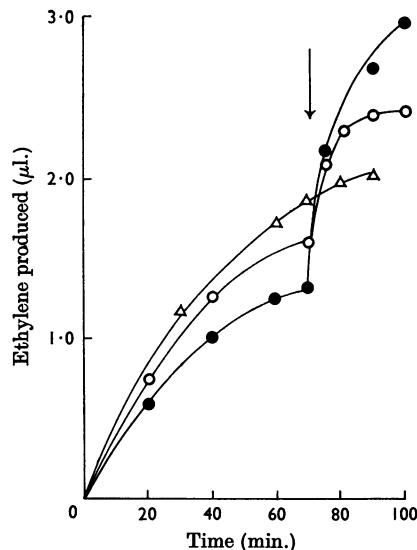


Fig. 3. Effect of Cu^{2+} (10 mM), Cu^+ (10 mM) or ascorbate (10 mM) in reviving the reaction initiated by H_2O_2 . For 60 min. the three systems contained phosphate buffer, pH 6.8 (60 mM), methionine (1 mM), H_2O_2 (5 mM) and Cu^{2+} (1 mM) in a total volume of 5 ml. Additions after 60 min. of reaction (indicated by \downarrow) were: Δ , Cu^{2+} (10 mM); \circ , Cu^+ (10 mM); \bullet , ascorbate (10 mM); the additions of the copper salts and ascorbate were made in saturated KCl solutions. The flasks were incubated at 30° with shaking.

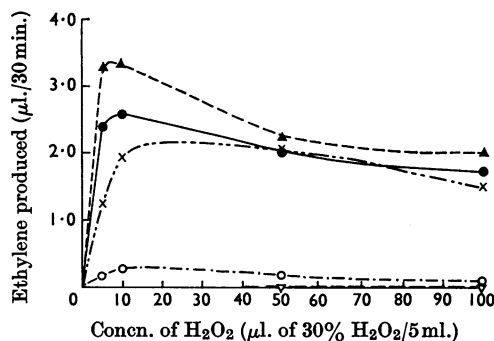


Fig. 4. Ethylene production as related to Cu^{2+} concentration, in the absence of ascorbate, at various concentrations of H_2O_2 . All reaction mixtures contained phosphate buffer, pH 6.8 (60 mM). The concentrations of Cu^{2+} used were: \times , 100 mM; \bullet , 10 mM; Δ , 1 mM; \circ , 0.1 mM; ∇ , 0.01 mM. The flasks were incubated at 30° for 30 min. with shaking.

This Cu^{2+} concentration is actually near optimum regardless of the amount of hydrogen peroxide used (Fig. 4). Ascorbate concentrations varying from 2 to 10 mM can serve best in the reaction with hydrogen peroxide, in the absence of added Cu^{2+}

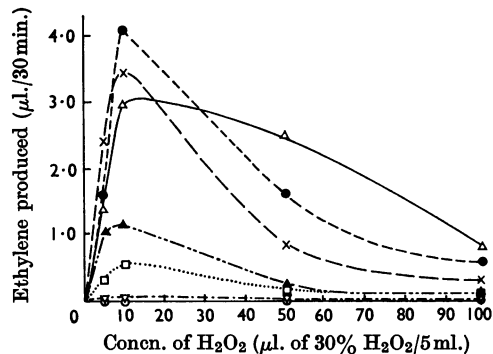


Fig. 5. Ethylene production as related to ascorbate concentration, in the absence of added Cu^{2+} or Cu^+ , at various concentrations of H_2O_2 . Contaminating Cu^{2+} , introduced with phosphate buffer, was at an approximate concentration of 0.01–0.1 μM . All reaction mixtures contained phosphate buffer, pH 6.8 (60 mM). The concentrations of ascorbate used were: \square , 100 mM; \triangle , 10 mM; \bullet , 5 mM; \times , 2 mM; \blacktriangle , 1 mM; ∇ , 0.1 mM. The flasks were incubated at 30° with shaking.

(Fig. 5). Apparently, very high concentrations of ascorbate provide an overwhelming reducing atmosphere, which prevents the action of hydrogen peroxide. With both Cu^{2+} and ascorbate present the optimum concentration of hydrogen peroxide is 10–17 mM (about 10 μl . of 30% hydrogen peroxide/5 ml.). This indicates that excessive hydrogen peroxide (above 17 mM) tends to inhibit the reaction and that concentrations below 8 mM (5 μl . of 30% hydrogen peroxide/5 ml.) cannot initiate the ethylene-forming reaction in the system lacking either ascorbate or added Cu^{2+} . These effects of low and high concentrations of hydrogen peroxide on the ethylene reaction may be concerned with formation of Cu^+ , which requires a minimum concentration of hydrogen peroxide, but excessive amounts of hydrogen peroxide tend to oxidize Cu^+ and thereby inhibit the reaction.

The fact that relatively high concentrations of Cu^{2+} and hydrogen peroxide are required in the 'abbreviated' ethylene-forming system, as compared with the usual model system, suggests that the production of ethylene may proceed via two mechanisms that involve high and low concentrations of hydrogen peroxide. The usual model system, which requires both ascorbate and Cu^{2+} , operates with low concentrations of hydrogen peroxide, generated endogenously, and can utilize Cu^{2+} in concentrations as low as 0.1 mM. The 'abbreviated' system operates at relatively high concentrations of hydrogen peroxide (17 mM) and requires either Cu^{2+} or ascorbate at 1 mM or higher. Additional evidence suggesting that two systems

Table 4. *Methional as substrate in the model system*

Flasks contained methionine or methional (1 mM), phosphate buffer, pH 6.8 (60 mM), Cu^{2+} (1 mM), ascorbate (10 mM) and, where indicated, semicarbazide hydrochloride (1 mM) in a total volume of 5 ml. Flasks were incubated at 30° for 30 min. with shaking.

Flask contents	Ethylene produced ($\mu\text{l./30 min.}$)
(1) Phosphate buffer + methionine + Cu^{2+} + ascorbate	3.00
(2) Phosphate buffer + methional + Cu^{2+} + ascorbate	19.17
(3) Phosphate buffer + methional + Cu^{2+} + ascorbate + semicarbazide	6.00
(4) Phosphate buffer + methional* + semicarbazide* + Cu^{2+} + ascorbate	2.70

* Methional and semicarbazide were incubated for 40 min. before phosphate buffer, Cu^{2+} and ascorbate were added.

are operating may be deduced from the results in Tables 3 and 4. The ethylene formed in the model system, with hydrogen peroxide at 17 mM [(10) in Table 3], is approximately equal to the sum of the ethylene formed in the usual model system (no hydrogen peroxide) [(1) in Table 4] and that produced in the 'abbreviated' system with high concentrations of hydrogen peroxide containing either ascorbate or Cu^{2+} [(3) and (8) in Table 3]. This suggests that the increase in ethylene formed in the model system on the addition of hydrogen peroxide is due to the operation of an additional ethylene-forming system.

Evidence for an intermediate thiol compound. At low concentrations of Cu^{2+} (0.01 mM) there is a rapid decline in ethylene production (Fig. 6a) and in the oxidation of ascorbate (Fig. 6b) in the model system. The decline in oxidation of ascorbate is related to the presence of methionine and therefore is associated with the ethylene-forming reaction. In the presence of hydrogen peroxide or with increasing concentrations of Cu^{2+} no such decline is seen (Figs. 6 and 7).

Inactivation of Cu^{2+} as a result of chelation by a product of the reaction provides an explanation for the decline in ethylene formation and cessation in oxidation of ascorbate at low Cu^{2+} concentrations (0.01 mM). The stimulation by hydrogen peroxide in this reaction (Fig. 6) may be due to splitting of the Cu^{2+} -chelate compound, thus releasing Cu^{2+} to function in the reaction. Hydrogen peroxide can also initiate the ethylene-forming reaction in the absence of added Cu^{2+} but in the presence of ascorbate, by using minute traces of contaminating Cu^{2+} , as indicated above [(3) in Table 3]. Inactiva-

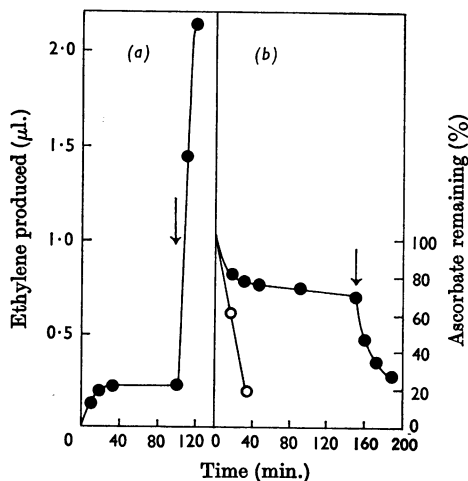


Fig. 6. Reaction inactivation in the system containing Cu^{2+} and ascorbate, and its reversal by H_2O_2 . The flasks contained phosphate buffer, pH 6.8 (60 mM), Cu^{2+} (0.01 mM) and ascorbate (10 mM), with (●) or without (○) methionine (1 mM), in a total volume of 5 ml. The arrow (↓) indicates H_2O_2 (10 mM) added. The flasks were incubated at 30° with shaking. (a) Ethylene produced; (b) ascorbate remaining.

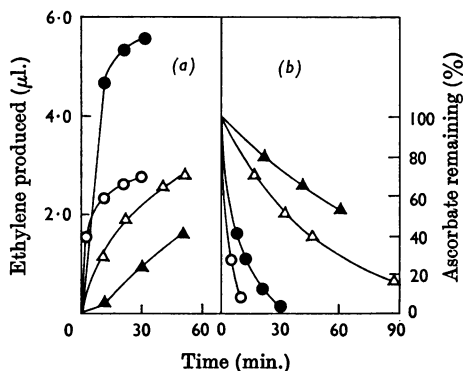


Fig. 7. Relationship between the rapid production of ethylene, oxidation of ascorbate and concentration of Cu^{2+} in the model system. The flasks contained phosphate buffer, pH 6.8 (60 mM), methionine (1 mM), ascorbate (10 mM), H_2O_2 (10 mM) and Cu^{2+} as indicated in a total volume of 5 ml. The concentrations of Cu^{2+} used were: ○, 1 mM; ●, 0.1 mM; △, 0.01 mM; ▲, 0.001 mM. The flasks were incubated at 30° with shaking. (a) Ethylene produced; (b) ascorbate remaining.

tion, in the model system with low Cu^{2+} concentrations, may also be prevented by simply increasing the concentration of Cu^{2+} so that it is never lowered excessively by the chelating compound formed during the reaction. Finally, inactivation can be

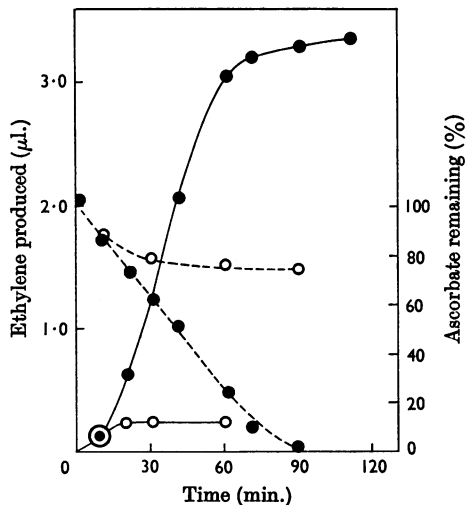


Fig. 8. Prevention of reaction inactivation in the Cu^{2+} -ascorbate system by p -chloromercuribenzoate. The flasks contained phosphate buffer, pH 6.8 (60 mM), methionine (1 mM), Cu^{2+} (0.01 mM) and ascorbate (10 mM), with (●) or without (○) p -chloromercuribenzoate. The flasks were incubated at 30° with shaking. —, Ethylene produced; ----, ascorbate remaining.

prevented by the addition of thiol reagents, e.g. p -chloromercuribenzoate and N -ethylmaleimide, which suggests that the chelating agent is a thiol (Fig. 8).

Direct evidence to show that a thiol is formed during the reaction was provided by analysis of thiol formed during the reaction, by using the S -nitroso reaction described by Liddell & Saville (1959). It was found (Fig. 9) that a thiol was rapidly formed but decreased in amount as the reaction progressed. Formation of the thiol was correlated with the initial formation of ethylene.

Methional as an intermediate. When methional was used in the model system, in the place of methionine, the production of ethylene increased more than sixfold (Table 4). This suggests that methional, which theoretically can be derived from methionine by decarboxylation and oxidative deamination, may be an intermediate in the methionine ethylene-forming system. It has not been possible to trap and identify methional as an intermediate in the methionine system, although the aldehyde reagents, semicarbazide and sodium bisulphite, inhibit the methionine model system by about 10%. However, the aldehyde reagents severely inhibit the methional ethylene-forming system [(3) and (4) in Table 4].

If methional is intermediate between methionine and ethylene, it is likely that the rate of production

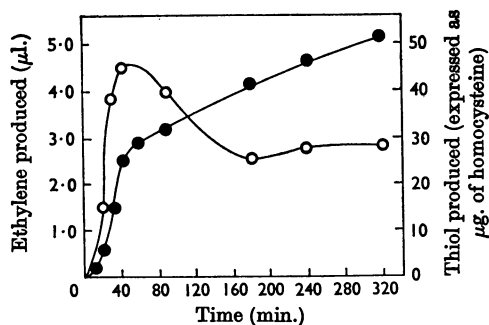


Fig. 9. Production of a thiol in the reaction with Cu^{2+} and ascorbate. The flasks contained phosphate buffer, pH 6.8 (60 mm), methionine (10 mm), ascorbate (10 mm) and Cu^{2+} (1 mm) in a total volume of 5 ml. The flasks were incubated at 30° with shaking. ●, Ethylene produced; ○, thiol produced.

of ethylene from the intermediate methional formed during the reaction is greater than the rate of formation of its semicarbazide or bisulphite addition product. This can explain the inability of the aldehyde reagents to block the reaction significantly in the methionine system.

There are similarities and differences between the methionine model system and the methional model system. In both systems an oxygen environment is necessary in the absence of hydrogen peroxide, except when Cu^+ is used directly in the reaction [(4) in Table 5]. One striking difference between the methionine and methional systems is the sizeable reaction of methional with hydrogen peroxide in the absence of added Cu^{2+} or ascorbate. Apparently, very small concentrations of contaminating Cu^{2+} can catalyse the conversion of methional into ethylene in the presence of hydrogen peroxide [(7), (8) and (9) in Table 5]. There is also much greater ethylene production in a reaction of methional with hydrogen peroxide and ascorbate than with hydrogen peroxide and Cu^{2+} . This, again, is different from the methionine system, wherein such reactions yield similar quantities of ethylene. Another difference concerns the relatively small increase in ethylene production in the complete methional system on the addition of hydrogen peroxide [(10) and (16) in Table 5]. With the methionine model system, ethylene production is approximately doubled on the addition of hydrogen peroxide, but with methional it is only increased by 10–15%. These differences between methional and methionine must be derived from the much greater reactivity of methional in this reaction. Apparently, the ceiling in ethylene production, under the conditions of the reaction, is approached sooner with methional in the absence of hydrogen peroxide, as compared with methionine.

Table 5. Effect of copper ions (Cu^{2+} and Cu^+), ascorbate and hydrogen peroxide on the methional model system

Flasks contained phosphate buffer, pH 6.8 (60 mm), methional (1.0 mm) and, where indicated, Cu^{2+} or CuCl (1 mm), ascorbate (10 mm), H_2O_2 (17 mm) and EDTA (0.01 mm) in a total volume of 5 ml. Flasks were incubated at 30° for 30 min. with shaking.

Flask contents	Atmosphere	Ethylene produced ($\mu\text{l.}/30\text{ min.}$)
(1) Phosphate buffer + methional + Cu^{2+}	Air	0.00
(2) Phosphate buffer + methional + Cu^{2+}	N_2	0.00
(3) Phosphate buffer + methional + Cu^+	Air	3.19
(4) Phosphate buffer + methional + Cu^+	N_2	2.86
(5) Phosphate buffer + methional + ascorbate	Air	0.32
(6) Phosphate buffer + methional + ascorbate	N_2	0.12
(7) Phosphate buffer + methional + H_2O_2	Air	0.65
(8) Phosphate buffer + methional + H_2O_2	N_2	1.86
(9) Phosphate buffer + methional + H_2O_2 + EDTA	Air	0.13
(10) Phosphate buffer + methional + Cu^{2+} + ascorbate	Air	18.15
(11) Phosphate buffer + methional + Cu^{2+} + ascorbate	N_2	0.48
(12) Phosphate buffer + methional + Cu^{2+} + H_2O_2	Air	3.74
(13) Phosphate buffer + methional + Cu^{2+} + H_2O_2	N_2	6.71
(14) Phosphate buffer + methional + ascorbate + H_2O_2	Air	9.79
(15) Phosphate buffer + methional + ascorbate + H_2O_2	N_2	10.72
(16) Phosphate buffer + methional + Cu^{2+} + ascorbate + H_2O_2	Air	21.41
(17) Phosphate buffer + methional + Cu^{2+} + ascorbate + H_2O_2	N_2	20.08

Other possible precursors of ethylene in the model system

The ability and inability of amino acids related to methionine, other substances metabolically related to methionine, and other amino acids, to form ethylene in the model system allows an analysis of the active structural components of the methionine molecule in the ethylene-forming reaction (Table 6).

Ethionine appears to be even more active a substrate than methionine, but homocysteine is relatively inactive. However, the *N*-ethylmaleimide derivative of homocysteine forms ethylene at 68% of the rate of the methionine system. The

Table 6. *Relative efficiency of ethylene production from methionine, methyl donors and other allied compounds*

Flasks contained substrate (1mm), Cu²⁺ (1.0mm), ascorbate (10mm) and phosphate buffer, pH 6.8 (60mm), in a total volume of 5ml. Flasks were incubated at 30° for 30 min. with shaking.

Substrate	Ethylene produced (% of that from methionine)
(1) L-Methionine	100
(2) DL-Ethionine	134
(3) DL-Ornithine	0
(4) Choline	1
(5) Betaine	1
(6) DL-Homocysteine	10
(7) L-Homocystine	0
(8) <i>N</i> -Ethylmaleimide derivative of DL-homocysteine	68
(9) Methionine sulphoxide	12
(10) Methionine sulphone	4
(11) L-Cysteine	0
(12) L-Cystine	0
(13) <i>N</i> -Ethylmaleimide derivative of L-cysteine	0
(14) <i>S</i> -Methylcysteine	0
(15) DL-Leucine	0
(16) DL-Isoleucine	0
(17) DL-Norleucine	0
(18) α -Aminobutyric acid	0
(19) β -Mercaptopropionic acid	0
(20) DL-Homoserine	6
(21) DL-Serine	0

Cu²⁺-chelating abilities of homocysteine are responsible for its inactivity in the reaction.

The methyl-donor characteristics of methionine appear not to be involved in this system, since methyl donors such as betaine and choline are inactive. *S*-Methylcysteine is also inactive, indicating the necessity for a C₂ link between the α -carbon and the sulphur atom in a reactive molecule. Homocystine is also inactive in the model system, but can be activated with hydrogen peroxide. This indicates the need for a less restricted sulphur atom at one end of the molecule. Both methionine sulphoxide and methionine sulphone are also inactive, again indicating the necessity for an unencumbered sulphur atom, except for the methyl or ethyl group, at the terminal end of the molecule.

Amino acids such as cysteine, cystine, leucine, serine, homoserine, ornithine and α -aminobutyric acid, and β -mercaptopropionic acid, are all impotent as sources of ethylene in the model system. It appears, therefore, that the molecular structure required for ethylene formation in this system is a -CH₂·CH₂- grouping in the centre of the molecule, with one end attached to a sulphur atom linked to a

methyl group or something similar, but otherwise unencumbered, and the other end attached to an aldehyde group or an acetyl group linked to an amino or hydroxy moiety.

Tracer studies

To determine which carbon atoms of the methionine molecule are converted into ethylene during the reaction, methionine labelled with ¹⁴C in the *S*-methyl carbon, the carboxy carbon, C-2 or C-3 and C-4 was used as substrate in the model system. Labelling in ethylene, carbon dioxide and other gases formed during the reaction was determined. These results (Table 7) show that significant labelling of ethylene was observed only with [3,4-¹⁴C₂]methionine as substrate.

[carboxy-¹⁴C]Methionine gave rise to considerable radioactivity in the gaseous products of the reaction; however, this was attributed to ¹⁴CO₂, since most of the radioactivity disappeared when the reaction was carried out in the presence of potassium hydroxide. With [2-¹⁴C]methionine as substrate there was also some ¹⁴CO₂ produced. Again, in this case the difference in radioactivity with and without potassium hydroxide implicates carbon dioxide as the gas in which virtually all of the ¹⁴C resides. However, much less ¹⁴CO₂ was obtained from [2-¹⁴C]methionine than from [carboxy-¹⁴C]methionine. Radhakrishnan & Meister (1957) have shown that amino acids can be decarboxylated and deaminated in the presence of hydrogen peroxide and Cu²⁺. This is the Strecker degradation reaction (Schönberg & Moubacher, 1952). Since the catalysts, Cu²⁺ and hydrogen peroxide, are either present or formed in the methionine model system, the first product after decarboxylation and deamination may well be methional, whose role as an intermediate in this system has been suggested above.

[Me-¹⁴C]Methionine gave rise to some radioactivity in gaseous products of the reaction. None of this radioactivity could be associated with meaningful amounts of ethylene, but could be related to small quantities of non-ethylenic volatile compounds that were not absorbed by mercuric perchlorate and are listed as 'other gases' in Table 7. These 'other gases' may be methane or methanethiol, since the radioactivity of the substrate resides in its methyl group and cleavage of the end group could occur and produce these compounds. However, the nature of the so-called 'other gases' has not yet been definitely established, though small quantities of methane are always observed as products of this reaction.

When [3,4-¹⁴C₂]methionine was used as substrate in the model system significant labelling of ethylene was observed (Table 7). Approximately equal

Table 7. *Radioactivity in gases from ¹⁴C-labelled methionine*

Model systems contained unlabelled or ¹⁴C-labelled methionine (2.5 μ C/flask) (1 mM), Cu²⁺ (1 mM), ascorbate (10 mM) and phosphate buffer, pH 6.8 (60 mM), in a total volume of 5 ml. Flasks were incubated at 30° for 1 hr. Warburg flasks, with KOH in side arms where indicated, were used for incubating the reaction mixtures.

Model system	Gas formation (μ moles/hr.)		Radioactivity (counts/min.)		
	Methane	Ethylene	Ethylene	CO ₂	Other gases
Expt. 1					
(1) Unlabelled L-methionine + KOH	14	67	8	—	8
(2) Unlabelled L-methionine — KOH	12	75	9	—	12
(3) L-[Me- ¹⁴ C]Methionine + KOH	13	67	17	—	2868
(4) L-[Me- ¹⁴ C]Methionine — KOH	13	67	18	—	2588
(5) DL-[2- ¹⁴ C]Methionine + KOH	13	53	23	—	12
(6) DL-[2- ¹⁴ C]Methionine — KOH	13	58	35	2160	12
Expt. 2					
(1) Unlabelled L-methionine + KOH	—	60	9	—	10
(2) Unlabelled L-methionine — KOH	—	62	10	—	12
(3) DL-[carboxy- ¹⁴ C]Methionine + KOH	—	76	15	—	18
(4) DL-[carboxy- ¹⁴ C]Methionine — KOH	—	71	15	21204	18
Expt. 3					
(1) Unlabelled L-methionine + KOH	10	69	9	—	11
(2) Unlabelled L-methionine — KOH	9	56	14	—	8
(3) DL-[3,4- ¹⁴ C ₂]Methionine + KOH	9	61	4776	—	13
(4) DL-[3,4- ¹⁴ C ₂]Methionine — KOH	9	55	5208	—	37

quantities of radioactive ethylene were formed in the reaction in the presence or absence of potassium hydroxide. The ethylene/methionine specific activity ratio was approx. 1. We must conclude from these experiments with ¹⁴C-labelled methionine that ethylene is not derived from the methyl carbon, the carboxy carbon, or C-2, but that ethylene is derived from C-3 and C-4, the two carbon atoms adjacent to the sulphur atom of methionine. These two carbon atoms were also implicated as precursors of ethylene in experiments in which the ethylene-forming activity of various substrates was assessed (Table 6).

Other products of the reaction

Sulphoxide and sulphone analogues. The methionine reaction mixture was chromatographed on paper in butan-1-ol-acetic acid-water (12:3:5, by vol.) or in phenol-water-butan-1-ol (12:3:10, w/v/v). The most obvious substance in the reaction mixture after 30–60 min., as detected by the ninhydrin reagent, was methionine sulphoxide. On longer incubations (3–16 hr.) methionine sulphone was the main product. The spot for methionine was faint, suggesting that most of it was converted into the sulphoxide or sulphone analogue. There was also a very faint spot for homocystine in some of the chromatograms. Analysis with the amino acid analyser, after 3 hr. incubation of the

model system with an extra addition of ascorbate (10 mM) each hour, revealed that approx. 1.75 μ -moles of the original 5 μ moles of methionine could be accounted for as methionine sulphone (1.58 μ -moles), homocystine (0.06 μ mole), homocysteic acid (0.04 μ mole) and residual methionine (0.05 μ mole). Since about 1.0 μ mole of ethylene, which is assumed to be equivalent to 1 μ mole of methionine, was produced in this reaction, there is about 2.25 μ -moles equivalent of methionine unaccounted for. Obviously, other products were formed that were not detected with ninhydrin reagent.

Sulphides, thiols, disulphides and acrolein. Additional products of the reaction detected were acrolein (CH₂:CH·CHO), ethyl methyl sulphide (CH₃·S·CH₂·CH₃), dimethyl disulphide (CH₃·S·S·CH₃) and a small amount of methanethiol (CH₃·SH). These were tentatively identified by gas chromatography after concentration in an enrichment trap, as described above. Since the detailed reaction mechanisms by which methionine is degraded are not known, the exact origin of many of the compounds is unknown. Dimethyl disulphide may arise from the oxidation of methanethiol, and ethyl methyl sulphide from the oxidation and decarboxylation of methional, which was implicated above as an intermediate in the model system. Ballance (1961) detected similar breakdown products in the degradation of methional by ninhydrin at elevated temperatures.

Although both acrolein and ethyl methyl sulphide may be products of the ethylene-forming reaction, and both yield ethylene in the model system, the rates of production of ethylene from either compound are only about one-quarter to one-half that observed with methionine in equivalent concentrations. On this account it seems difficult to conceive of these substances as direct intermediates in the pathway leading to ethylene. We have also found (1) that the *N*-ethylmaleimide derivative of β -mercaptopropionic acid ($\text{HS}\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{CO}_2\text{H}$) is a very poor substrate for ethylene in the model system and (2) that methylthiopropionic acid ($\text{CH}_3\cdot\text{S}\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{CO}_2\text{H}$), produced by the oxidation of the aldehydic group of methional with silver oxide (Mitchell & Smith, 1950), produces little or no ethylene. It seems possible that decomposition of methional occurs simultaneously with the oxidation of the aldehydic group, the precise mechanism of which must be left for future investigation. The products, acrolein, ethyl methyl sulphide, dimethyl disulphide etc., may well be produced in this system by alternative degradation pathways that do not always lead to ethylene.

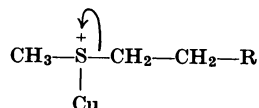
The results given in Table 8 show that methionine sulphone is virtually useless as a substrate for ethylene in the Cu^{2+} -catalysed reaction system, and that methionine sulfoxide is only slightly better. Therefore, the formation of sulfoxides or sulphones will significantly lower the production of ethylene in the methionine model system. However, methionine sulfoxide produces a relatively large amount of methane (Table 8), which may explain the origin of methane in the experiments with the methionine model system. Since methane production is noticeably increased when hydrogen peroxide is added to the methionine system, a greater con-

version into methionine sulfoxide is indicated (Dent, 1948). However, the addition of hydrogen peroxide does not significantly stimulate ethylene production in the model system with either methionine sulfoxide or methionine sulphone as substrate.

DISCUSSION

The reaction of methionine with a Cu^{2+} -ascorbate system, so far as can be ascertained, has not been previously described. It takes place at approx. 30° and, among other things, gives rise to hydrocarbon gaseous products. Ethylene is the main hydrocarbon gas formed but a small amount of methane and minute amounts of ethane are also produced. Other products of the reaction, such as acrolein, ethyl methyl sulphide and dimethyl disulphide, were also detected but quantitative aspects of their production are not yet known.

The role of Cu^{2+} in this reaction is unique and may be attributed to the facility with which Cu^+ can produce hydrogen peroxide (Mapson, 1941). However, Cu^+ appears to have an additional function which is associated more directly with the actual degradation of methionine. The formation of ethylene from methionine requires a breaking of the $\text{C}_{(4)}\text{-S}$ bond. Since Cu^+ forms complexes with sulphur it is reasonable to assume that fission of the C-S bond will be aided by complex-formation as follows:



Support for this mechanism may be derived from the fact that virtually no ethylene is formed by either methionine sulfoxide or methionine sulphone, when these are substituted as precursors in the methionine- Cu^{2+} -ascorbate system. In the aforementioned oxidized methionine compounds the Cu^+ will only bind to the sulphur atom with some difficulty or not at all. Methionine sulfoxide (in which the sulphur atom is encumbered by only one oxygen atom, as compared with methionine) forms considerably more methane than methionine does in the presence of Cu^+ , which suggests that the break in the C-S bond occurs more readily between the sulphur atom and the methyl group. Methionine sulphone (in which the sulphur atom is encumbered by two oxygen atoms, compared with methionine) forms no methane or ethylene, suggesting that there is no instability in the C-S-C bonds. Binding of the sulphur atoms in *L*-homocystine may also explain its inactivity as an ethylene precursor in the Cu^+ -catalysed reaction. In this case, the combination of Cu^+ with homocystine may form a stable adduct

Table 8. *Production of ethylene from methionine sulfoxide and methionine sulphone*

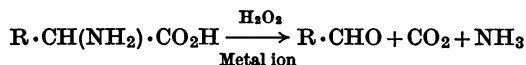
Flasks contained phosphate buffer, pH 6.8 (60 mm), methionine, methionine sulphone or methionine sulfoxide (1.0 mm), Cu^{2+} (1 mm) and ascorbate (10 mm) in a total volume of 5 ml. Where used, the concentration of H_2O_2 was 17.5 mm. Flasks were incubated at 30° for 30 min. with shaking.

Substrate	Gas formation ($\mu\text{l.}/30\text{min.}$)	
	Methane	Ethylene
(1) <i>L</i> -Methionine	0.04	3.24
(2) <i>DL</i> -Methionine sulphone	0.00	0.01
(3) <i>L</i> -Methionine <i>DL</i> -sulfoxide	0.41	0.04
(4) <i>DL</i> -Methionine sulphone + H_2O_2	0.07	0.61
(5) <i>L</i> -Methionine <i>DL</i> -sulfoxide + H_2O_2	0.58	0.22

that will not cause a splitting of the C-S bonds, the necessary prerequisite for ethylene formation.

Ethylene obtained from methionine, when ascorbate and hydrogen peroxide react with it in the absence of added Cu^{2+} , is due almost entirely to contaminating Cu^{2+} introduced with phosphate buffer. There is, however, a residual amount of activity that cannot be eliminated by EDTA or other chelating agents. This residual activity in the ascorbate-hydrogen peroxide system in the presence of EDTA is attributed to dehydroascorbate, which is listed as a ketone reagent capable of effecting Strecker degradation of α -amino acids (Schönberg & Moubacher, 1952).

The term Strecker degradation refers to the decomposition of α -amino acids, with any degrading agent, to aldehydes and ketones containing one carbon atom less/mol. (Schönberg & Moubacher, 1952). Hydrogen peroxide, in the presence of Fe^{2+} , degrades α -amino acids, such as glycine, at room temperature (Dakin, 1906). The following general equation is suggested for the Strecker degradation of α -amino acids by hydrogen peroxide and reduced metal ions (Schönberg & Moubacher, 1952):



The degradation of methionine by Cu^{2+} in the presence of ascorbate initially must represent just such a Strecker degradation. The methional formed is subject to further rapid degradation by the original reactants, Cu^{2+} and hydrogen peroxide, and this leads to the production of ethylene. The tracer experiments provide evidence that carbon dioxide is formed during the methionine degradation, and this is further support for a Strecker degradation mechanism. These experiments with ^{14}C -labelled methionine show that C-3 and C-4 are the precursors of the ethylene molecule.

The formation of ethylene in the methionine model system can be compared with that in the linolenate model system (Lieberman & Mapson, 1964). Both require Cu^+ and involve either a strong oxidizing agent, such as hydrogen peroxide,

or an oxidized form of the substrate (peroxidized linolenate). The methionine system produces about 3-5 times as much ethylene/mol. of substrate as does the linolenate system in equivalent time period. Perhaps such comparisons are unwarranted at present, since in addition to ethylene a series of other hydrocarbon gases are also formed in the linolenate system. The significant fact is that Cu^+ and an oxidizing agent, or an oxidized or activated form of the substrate, are necessary for ethylene production in model systems with two very different kinds of substrate molecules.

Evidence has been obtained (M. Lieberman, L. W. Mapson, A. T. Kunishi & D. A. Wardale, unpublished work) that methionine can stimulate the biosynthesis of ethylene by apple tissue slices, and that chelating agents that tend to bind Cu^{2+} inhibit ethylene production by these tissues. This suggests the possibility that methionine is a precursor of ethylene in tissues and that the enzymic system associated with the biosynthesis of ethylene involves a copper enzyme, perhaps a peroxidase.

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